HEART REGENERATION AFTER MIOCARDIAL INFARCTION USING SYNTHETIC BIOMATERIALS

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Abstract:
Myocardial infarction causes almost 7.3 million deaths each year worldwide. However, current treatments are more palliative than curative. Presently, cell and protein therapies are considered the most promising alternative treatments. Clinical trials performed until now have demonstrated that these therapies are limited by protein short half-life and by low transplanted cell survival rate, prompting the development of novel cell and protein delivery systems able to overcome such limitations. In this review we discuss the advances made in the last 10 years in the emerging field of cardiac repair using biomaterial-based delivery systems with focus on the progress made on preclinical *in vivo* studies. Then, we focus in cardiac tissue engineering approaches, and how the incorporation of both cells and proteins together into biomaterials has opened new horizons in the myocardial infarction treatment. Finally, the ongoing challenges and the perspectives for future work in cardiac tissue engineering will also be discussed.

Key words: myocardial infarction, cell therapy, protein therapy, clinical trials, synthetic biomaterials, delivery systems, tissue engineering.

1. INTRODUCTION

1.1. Myocardial infarction and current treatments

Myocardial infarction (MI) remains a leading cause of morbidity and mortality worldwide, being responsible for nearly 7.3 million deaths each year. Moreover, as the World Health Organization highlighted in the last "Global Atlas on cardiovascular disease prevention and control" report [1], the number of deaths is expected to increase within the next decades due to the rising prevalence of the key risk factors for this pathology, such as behavioral and metabolic factors.

MI is principally caused by the occlusion of a coronary artery due to atherosclerotic and thrombotic processes, with the consequent reduction of the blood flow to the heart muscle. That loss of blood supply to the myocardium induces functional and morphological consequences. First, ischemic conditions lead to cardiomyocyte (CMC) death by necrotic or apoptotic processes, generating an infarcted area and causing a defect in contractile function. As a consequence, progressive and negative left ventricle (LV) remodeling and scar tissue formation take place [2]. These changes affect the ventricular chamber geometry, leading to the emergence of a larger, thinner and more spherical heart shape. Although the collagen-rich scar provides a rapid solution that avoids total LV wall disintegration, progression of the MI event often culminate in total heart failure and death [2]. A schematic representation of MI development with the principal steps is shown in Figure 1.

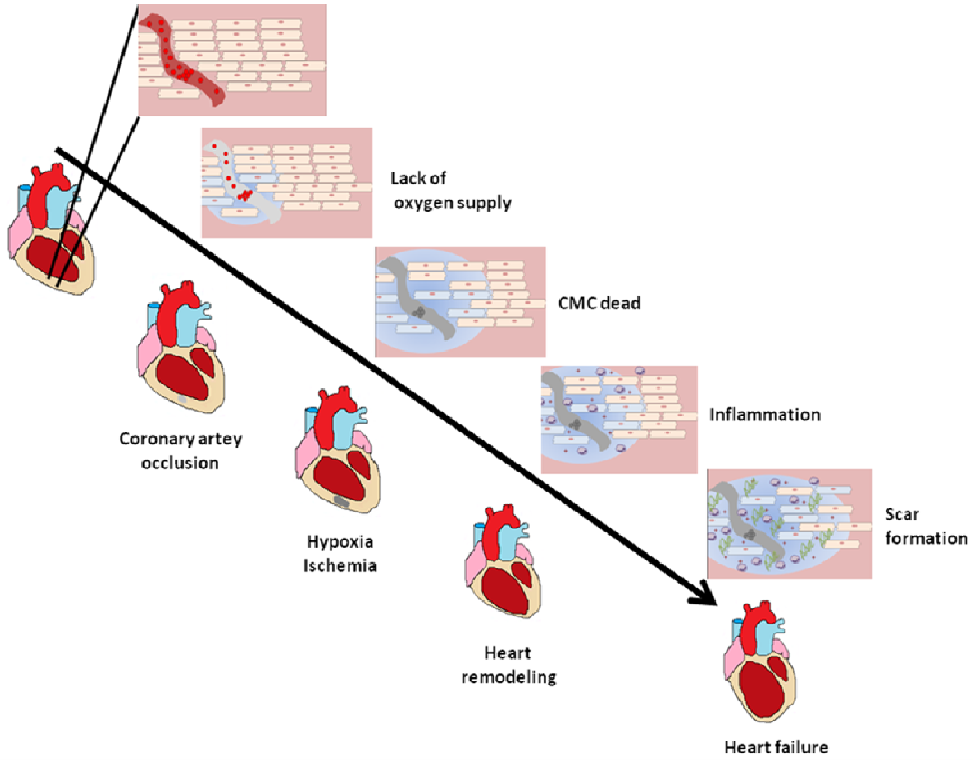

Figure 1: Schematic representation of MI development including the principal steps. After a coronary artery occlusion, the heart gradually loses its function and suffers a negative remodeling that ends in total heart failure. The black arrow indicates the progression of the pathology.

Death from MI could be prevented by accurate early-stage diagnosis and proper subsequent proper treatment [3]. The more quickly the blood flow is restored, the better the outlook. Current therapies include surgical procedures such as coronary bypass, balloon angioplasty, stents and heart transplant as a last option [4]. Surgical interventions are generally combined/complemented with pharmacological treatments in order to improve patient outcomes [5]. However, although conventional interventions are useful in mitigating MI symptoms [6,7], they cannot repair the infarcted tissue, an so cardiac dysfunction remains an issue [8]. In view of the fact that current treatments are not able to regenerate the cardiac tissue and that the heart has shown limited post-natal cardiomyogenesis [9], patients who survive a MI might face

serious functional limitations for the rest of their lives, which leads to secondary complications that impair their quality of life and place a mayor annual economic burden on the country [10].

1.2. New therapeutic strategies under investigation for myocardial infarction

As already stated above, conventional treatments are not enough to deal with functional and economic complications derived from MI and many aspects of the treatment for this pathology remain challenging. Therefore, in recent decades there has been a great research effort aimed at finding new alternative therapies for MI, focusing on myocardial regeneration. In these investigations, angiogenesis, CMC proliferation and recruitment of stem cells (SCs) to enhance endogenous healing of the heart have played an essential role, since they are considered to be key factors for adequate post-ischemic repair [11,12]. The advent of new molecular and cellular targets together with advances in genomics, proteomics and other biotechnologies have led to the discovery of novel pharmaceutical compounds with the potential to definitively change MI treatment. This emerging class of substances include biological agents, genes, siRNAs, small molecules such as growth factors (GFs) and other therapeutics [13]. Among them, the ones that have shown the best results so far are cells and proteins [14,15]. In fact, exciting preclinical studies carried out to evaluate regenerative therapies for MI have prompted the initiation of clinical trials based on administration of SCs or GFs to the heart, as shown in the sections below.

1.2.1 Cell therapies for MI in clinical trials

Cell therapy relies on the administration of living cells for therapeutic purposes. Focusing on myocardial regeneration, it requires the administration of multipotent cells able to differentiate into the main cardiac cell lineages myocytes, vascular smooth muscle cells and endothelial cells [16] and to develop both CMCs and coronary vessels [17]. To date, the most popular cell candidates used to regenerate the damaged tissue include adipose derived stem cells (ADSCs), mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), endothelial progenitor cells (EPCs), bone marrow derived stem cells (BMSCs), induced pluripotent stem cells (iPS), cardiac progenitor cells (CPCs) and induced cardiomyocytes (iCMs) [18]. However, although several SCs have been tested in *in vitro* and *in vivo* preclinical studies with promising results [19], not too many SCs have reached clinical trials. In fact, only BMSCs, myoblasts, CPCs and ADSCs have been employed in clinical trials yielding both encouraging and disappointing results [20]. The discrepancies in the results of different clinical trials using the same cell source have led researches to investigate the key aspects that determine the success of cell therapy. A careful analysis of the available data shows that negative results may be fundamentally due to the poor permanence of the injected cells inside the tissue [21,22], since SCs therapeutic efficacy depend on their ability to survive in the hostile milieu of the damaged heart and to engraft within the myocardium [23]. Another critical point is the complexity of obtaining a reliable source of functional CMCs. Moreover, there are other aspects such as ideal cell type, source and dosing, route and time of delivery [24] and clinical trial design which should undergo further analysis to validate the safety and efficacy of cell therapy for MI [25]. In addition,

cardiovascular regeneration may not be identical among individuals, and there should be an optimal cardiac regeneration therapy for each patient [26]. In summary, although the outcomes of clinical trials performed so far have displayed promising results, the overall beneficial effects of SCs therapies are still relatively modest. Moreover, the fundamental mechanisms of SC-mediated repair are largely unknown and controversial. Interestingly, the slight improvement observed after cell administration is frequently due to the paracrine effect of the cells rather than to their differentiation [19,27]. Thus, bioactive factor secretion may mediate the improvement in cardiac remodeling, function and metabolism. The latest research trend in SC therapy for cardiac healing identified exosomes secreted by SCs as crucial mediators of cell therapy-induced regeneration [28].

1.2.2 Protein therapies for MI in clinical trials

In addition to cell therapy, the administration of GFs able to promote cardiac repair holds great promise as a therapy capable of contributing to myocardial regeneration. GFs are administered next to the damaged tissue with the aim of favoring angiogenesis, chemotaxis, SC differentiation, CMC survival and proliferation, reduction of apoptosis and remodeling [5]. First, it was reported that during MI evolution the administration of therapeutic GFs could help to enhance the endogenous angiogenic process [29], thereby improving cardiac function and recovery. Interestingly, more recently it has been demonstrated that GF administration also has effects on stimulating progenitor cell recruitment to the heart and on inducing differentiation the of SCs and existing CMCs [15]. In fact, the combination of all the three processes is mandatory for achieving the best possible heart regeneration. Therefore optimism about how protein-based approaches can be effective for cardiac regeneration and can avoid the fatal consequences of MI disorder has spread considerably in the last few years. Consequently, several GFs have been brought to clinical trials to test their therapeutic potential to regenerate the infarcted heart. This is the case of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), erythropoietin (EPO), hepatocyte growth factor (HGF), neuregulin (NRG), granulocyte-colony stimulating factor (G-CSF) and stromal cell-derived factor-1 (SDF-1) [5,30]. Again, as reported in clinical trials with cells, important but controversial results have been observed in clinical trials with GFs. By summarizing these results some interesting conclusions can be outlined. Firstly, the study designs vary considerably from one trial to another in terms of population, GF administered, route and dose of administration. Thus, a better definition of clinical trial requirements is needed in order to obtain more comparable results and conclusions. Secondly, a common drawback observed in all these trials is the low half-life of therapeutic proteins in the organism, which are rapidly degraded or removed from the site of injection. Therefore, the low efficacy and variable results reported so far might be attributed to this bioavailability issue. Thus, protein therapy needs to overcome those obstacles before it can attain clinical relevance.

1.2.3 Current challenges

Taking an overview of the aforementioned results, it can be gathered that SC and protein based therapies are potentially powerful strategies for treating MI. However,

only partial improvements have been achieved, and more research is needed to optimize such therapies. The advantages and challenges of cell and protein therapies are illustrated in Figure 2.

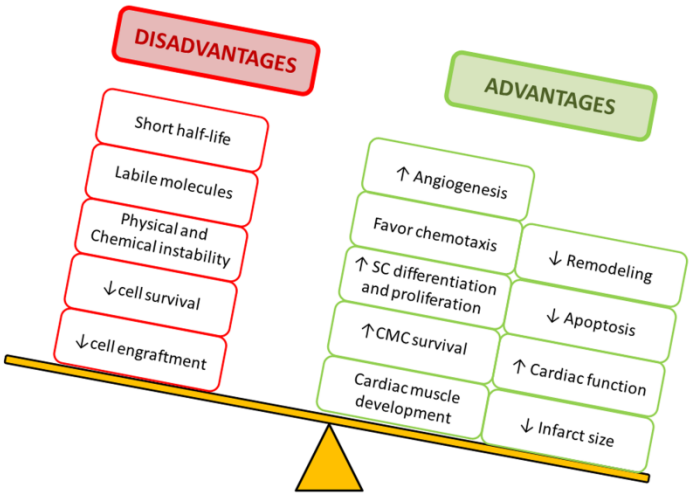

Figure 2: Advantages and challenges of cell and protein therapies.

Principally, enhancement of cell engraftment, integration and coupling in the tissue must be at the center of efforts in cell therapy, whereas in protein therapy, improvements in bioactivity half-life and stability of therapeutic proteins are the principal points in where deeper investigation is mandatory. It is necessary to improve the efficacy of these novel strategies to reach their full potential. Importantly, specialized delivery modalities are highly recommended to achieve these goals.

2. BIOMATERIALS TO ENHANCE CELL AND PROTEIN DELIVERY TO THE HEART

2.1. Biomaterials in cardiac repair

The development of new technologies that enable effective cardiac drug delivery would optimize cardiovascular treatment and would address the shortcomings of current and novel therapies. Biomaterials were developed to be used as medical devices for transplantation. However, the term biomaterial has evolved from simple implants to complex multifunctional interfaces with the body. The current definition proposed by the *European Society for Biomaterials* is a “material intended to interface with biological systems to evaluate, treat, augment or replace any tissue organ or function of the body” highlighting the role and importance of the material in influencing biological processes. Biomaterials should play a crucial role in the repair of the damaged heart. In a cardiac context, there are 4 ways in which biomaterials have shown to be useful (Figure 3):

a) The biomaterial by itself promotes cell migration or tissue regeneration. In this case, biomaterials reproduce some aspects of the natural cardiac tissue environment and encourage tissue regrowth. Biomaterials are used for general cardiac reconstruction, vascular grafts, pediatric shunts, etc. Synthetic materials, metals,

combinations of both and decellularized materials have been used for many years with significant success [31].

b) The biomaterial is used to encapsulate cells acting as an immunoisolation barrier. Encapsulation is one potential strategy to increase viable cell retention while facilitating paracrine effects. Synthetic biomaterials have evolved from polymers with no cell-recognition moieties to compounds mimicking the extracellular matrix, thus favoring cell-biomaterial interactions [32]. Nevertheless, they have not yet reached the cell viability and proliferation rates observed with natural biomaterials. Consequently, synthetic polymers are used in combination with natural compound or small peptide sequences in order to promote cell-biomaterial interactions for tissue regeneration [33].

c) The biomaterial is used as a matrix to support cell growth and integration. The biomaterial improves cell behavior due to the 3D environment as well as to the mechanical and signaling cues they provide to transplanted cells. These biomaterials are thus used as scaffolds. Several parameters must be taken into consideration in scaffold design to meet heart-specific requirements, such as shape, size and physical and mechanical properties [34].

d) The biomaterial is used as a controlled release reservoir to locally deliver bioactive molecules. Biomaterials can be used to prepare drug delivery systems (DDSs) that might provide protection from degradation to biomolecules (e.g. GFs, transcription factors, soluble paracrine factors) and a prolonged delivery. Thus, DDSs are able to decrease the amount of drug given to the patient, reducing serious side effects besides promoting cardiac repair. Each biomaterial, regarding its physico-chemical properties, provides a particular release profile. Thus, a specific biomaterial must be used for achieving the desired controlled release.

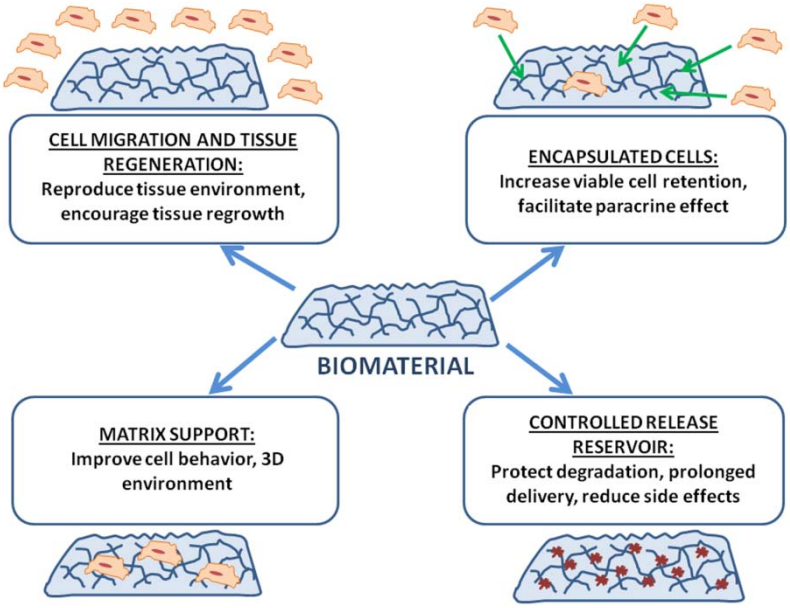

Figure 3: Principal benefits of biomaterials in cell and protein therapies.

2.2. Current studies using biomaterial-based delivery systems in heart regeneration

Biomaterial-based delivery systems are essential in enhancing the therapeutic outcomes of cells and proteins in cardiac tissue engineering. The number of studies using biomaterials in combination with cell and protein therapies has therefore increased exponentially over the last decade. This section provides an overview of the advances made in the last 10 years in the field of cardiac repair using biomaterial-based delivery systems with a focus on the progress made on preclinical *in vivo* studies done with synthetic biomaterials. We center on the use of synthetic biomaterials because they have been recently favored as cell and protein carriers. Nowadays there is a large list of synthetic biomaterials under investigation, including caprolactone, polyglycolic and polylactic acids, polyurethane and self-assembling peptides among others (Table 1). Each one has its own characteristics, but, in general all present important advantages over their natural counterparts. For instance, their physical, chemical, mechanical and biological properties can be modified, they can be produced uniformly in large quantities offering a multitude of possibilities. They also offer limited batch-to-batch variability. Concerning toxicity, nowadays synthetic biomaterials have reached similar safety levels to natural compounds, and their biocompatibility and biodegradability are well established in almost all cases (Table 1). A point that must be considered when designing biomaterial-based delivery systems for heart regeneration is the size of the DDS, since it could induce important side effects. On one hand, administration of "big" DDSs might favor tissue necrosis or hamper cardiac muscle contraction. On the other hand, very small DDSs could be rapidly phagocyted or cleared by the blood flow diffusing to other body organs. For instance, our preclinical studies using small (rat) and large (minipig) animal models of MI demonstrate that particles between 5 to 20 µm have the ideal size for intramyocardial injection. Smaller particles presented poor retention in the injected tissue, whereas injection of bigger particles resulted in damaged cardiac tissue [35–37].

Table 1: Most employed synthetic biomaterials used to prepare cardiac DDSs and their principal advantages and disadvantages.

Synthetic biomaterial	Advantages	Disadvantages
Caprolactone and derivatives	Non-toxic, tissue compatible, mechanical properties, modifiable nature, pH sensitivity	Difficult to synthesize, slow biodegradation [38,39]
Polyglycolic and polylactic acids and derivatives	Well established biodegradation and biocompatibility, extended release rates	Acidic environment during degradation, bulk erosion [39]
Polyurethane	Biocompatible, mechanical properties	Biodegradable only when copolymerized with other polymers, no conductivity [39]
Self-assembling RAD 16 peptides	Self-assembly properties, bioreabsorbable, designed 3D microenvironment	Unknown toxicity and side effects [40,41]

Carbon nanotubes	Excellent mechanical and electrical properties	Strong hydrophobicity, physicochemical properties related toxicity, expensive [42]
Polyketals	Biodegradable, non-immunogenic, neutral degradation products, acid sensitivity, low cost	Rapid macrophage uptake and biodegradation, complex synthesis [43]

2.2.1. Hydrogels

Injectable hydrogels are three-dimensional polymer networks extensively swollen in water (Figure 4) [44], and represent a powerful delivery system for cardiac repair, since they are tri-dimensional networks that mimic the extracellular matrix and reproduce the natural environment and, in addition, they can be administered using non-invasive techniques like cardiac catheterization, thanks to their liquid-gel controllable nature [45]. In accordance with this potential, hydrogels have been developed using a long list of biomaterials. Nowadays synthetic materials have achieved high degrees of biodegradation and biocompatibility, like their natural counterparts. In fact, cardiac administration of synthetic hydrogels has proved to be effective in terms of promoting contractile phenotype smooth muscle tissue formation [46,47], preventing LV remodeling and scar expansion and improving cardiac function [48–51]. Interestingly, the timing of administration seems to be important because hydrogels assumed markedly different morphologies that determined heart remodeling. Thus, very early time points may not be beneficial, whereas hydrogel injection one week after the infarct event results in positive remodeling and cardiac function improvements [50].

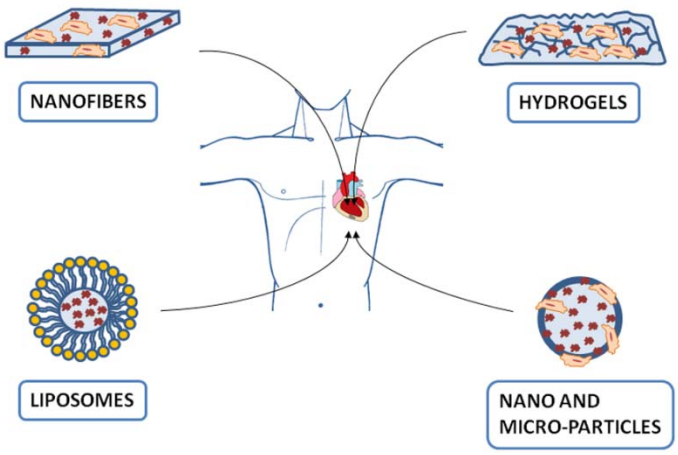

Figure 4: Main types of drug delivery systems made of biomaterials that are being investigated in combination with proteins and/or cells for treating myocardial infarction.

2.2.1.1. Hydrogels in cell-based therapies

The use of hydrogels as cell carriers to repair the heart is a relatively new strategy. The resulting network that hydrogels form can reproduce specific biological functionality of

the natural cardiac extracellular matrix and thus, seeded cells grow under conditions as similar as possible to those of the natural environment.

To examine the effect of cell delivery via hydrogels, Wall S.T. *et al*. developed a semi-interpenetrating hydrogel made of a crosslinked copolymer network of N-isopropylacrylamide and acrylic acid, interpenetrated with linear chains of polyacrylic acid with chemically tethered peptides combined with cell-surface integrin receptors for encouraging cellular attachment. The system was used as an assistive microenvironment for BMSC transplantation (2×10^5 cells per hydrogel) and tested in a mouse MI model. Six weeks after implantation, hydrogels were at the site of injection and GFP-BMSC could be detected. However, BMSC-hydrogel treatment did not show any improvement in ejection fraction (EF) and fractional shortening (FS) compared to non loaded hydrogels and free BMSC injection [52]. Concurrently, Li X.Y. *et al*. developed a crosslinked polymer hydrogel by mixing dextran-hydrophobic poly(e-caprolactone)-2-hydroxylethyl methacrylate chains with thermo-responsive poly(N-isopropylacrylamide) (NIPAM) chains. The biomimetic network was then combined with BMSC and placed onto the LV of rabbit MI model. A significant increase in cell engraftment 48 h after injection compared to free SC administration was observed. One month after treatment significant LV-EF preservation and attenuated LV dilatation accompanied by enhanced neovascular formation and prevented scar expansion were found in BMSC-hydrogel group compared to the rest of the groups [53].

Limited positive results have been reported with hydrogels prepared using only poly(ethylene-glycol) (PEG) [54]. However, several PEG copolymer hydrogels have shown benefits. For instance PEG has been combined with synthetic caprolactone by Wang T. *et al*. A triblock polymer was synthesized by mixing methoxy PEG and poly(caprolactone)-(dodecanedioic acid)–poly(caprolactone). BMSC ($2x10^7$) were resuspended in α-cyclodextrin solution, which was intramyocardially co-injected with the hydrogel solution in a rabbit MI model. BMSC-hydrogels significantly enhanced cardiac function and increased both cell retention and vessel density around the infarct, preventing scar expansion compared with cells injected alone 4 weeks after treatment [55]. PEG has also been combined with natural materials to formulate hydrogels for cell transplantation. Naturally occurring biomaterials allow for the appropriate cell–matrix interactions, thus favoring cell engraftment [56]. The first example is the study by Habib M. et al. who PEGylated bovine fibrinogen to synthesize a biocompatible matrix where neonatal rat ventricular CMCs ($3x10^6$) were seeded. Irgacure2959 photoinitiator was added to allow UV-light-activate *in situ* polymerization of the hydrogels after injection into the myocardium of rats suffering MI. Owing to the PEG additional crosslinkers, hydrogels permanence in the tissue varied from less than one month on the absence of additional PEG, to more than one month when additional PEG up to 2% was added. The combination of CMC and hydrogels resulted in a favorable effect on cardiac remodeling with a significant increase in FS and functional outcomes 30 days after treatment administration. Higher anterior wall thickness was also detected in the CMC-hydrogel group when compared to controls, and transplanted CMC were detected one month after administration inside the infarcted region [57]. In another approach Bearzi C. *et al*. synthesized a PEG-fibrinogen hydrogel using PEG-diacrylate as crosslinker and the photoinitiator Irgacure 2959 to control gelation. iPS ($5x10^6$) over-expressing placental growth factor (PIGF) and/or matrix

metalloproteinase 9 (MMP9, protease involved in vascularization and engraftment processes, $5x10^6$ cells) were combined with the hydrogels and tested in a mouse MI model. Animals that received iPS-hydrogel, regardless of whether cells over-expressed the therapeutic proteins or not, showed a significant increase in capillary density and cardiac function and a decrease in fibrotic and apoptotic indexes. Administration of SCs over-expressing both proteins resulted in better outcomes [58]. Positive results were also observed by Wang H. *et al*., who combined PEG with fumarate. In this case, APS/TEMED solution was used for controlling hydrogel gelation. ESC ($1x10^6$) were seeded and systems efficacy was tested in a rat MI model. 24 hours and 4 weeks after treatment a significant higher injected-cell population was detected in the infarcted tissue in ESC-hydrogel group compared to injection of cells alone. The heart area covered by transplanted cells was also significantly bigger when the hydrogel was used. Concerning cardiac parameters, echocardiography values, infarct size and arteriole/venule density were significantly improved in ESC-hydrogel group when compared to controls 4 weeks after treatment [59].

In addition to cardiac hydrogels based on PEG there is a growing interest on the development of hydrogels made from self-assembling peptides. Self-assembling is a process that is mediated by non-covalent interaction between molecules *via* ionic bonds, hydrogen bonding, hydrophobic interactions and van der Waal interactions [60]. Self-assembling peptides are normally 8-16 amino acids long and they are composed of alternating hydrophilic and hydrophobic residues that form a stable hydrogel of flexible nanofibers (NFs) upon exposure to physiological salt concentration or pH [61]. This is the case with the self-assembling polypeptide RAD16-II and its derivates. This polypeptide is able to spontaneously assemble into a stable three-dimensional NF scaffold that mimics natural extracellular matrix [62]. Davis M.E. *et al*. demonstrated that RADA16-II peptides rapidly gel when mixed with sterile sucrose solution. The resulting hydrogel created a microenvironment in the myocardium which promoted vascular cell recruitment and favored injected cell survival [63]. Then, Lin Y.D. *et al*. seeded BMSC (10^8) in such DDS and tested them in a pig MI model. BMSC-hydrogel injection resulted in significant higher improvement of cardiac function compared to other groups that was accompanied by a significant increase in transplanted cell retention and capillary density in the peri-infarct area. Similar significant results were observed regarding the scar length fibrosis area, which were reduced in animals treated with seeded hydrogels compared to other groups [64]. More evidence about the use of RADA16 peptides for cardiac implantation was given by Cui X.J. *et al*. Here, MSCs ($5x10^5$) were seeded on the polypeptide hydrogel and injected in a rat MI model [65]. Injected cells underwent myogenic differentiation in the infarct and peri-infarct regions 4 weeks after administration. Smaller infarct size, higher capillary density and improved global cardiac function were observed, with significant differences between animals treated with MSC-RADA16-hydrogel and the rest of the groups [66]. These encouraging results led Guo H.D. *et al*. to attach an RGDSP cell-adhesion motif to RADA16 peptide to enhance cell survival and differentiation and thereby to improve SC efficacy. $5x10^6$ BMSC were seeded in these systems and final constructs were tested in a rat MI model. The system protected SCs from apoptosis and necrosis processes present in the ischemic myocardium. Moreover, MSC survival rate, cardiac function and collagen deposition were improved in animals treated with RGDSP-MSC-hydrogels with respect to MSC-hydrogels group 4

weeks after treatment [67]. Tokunaga M. *et al*. further demonstrated the efficacy of RADA16 peptide as cell carrier in a mice MI model. In this study, Puramatrix^{TM}, a commercial variant of RADA16 peptide, was used to create self-assembling hydrogel that underwent gelation in presence of salts from the body. Authors seeded $2x10^4$ BMSC, SM, ADSC or CPC in such systems and injected them onto the infarcted area of a mouse MI model. 2 weeks after treatment CPC-hydrogel significantly attenuated infarct size expansion, improved echocardiography parameters and favored neoangiogenesis. Cell apoptosis was reduced when hydrogels were employed compared to free cell administration. These results suggest that CPCs are a promising cell source for preventing cardiac remodeling and dysfunction [68].

More recently, carbon nanotubes have been explored for cardiac delivery. They have good electrical conductivity and suitable and adaptable mechanical properties for cardiac application. A novel hydrogel made of carbon-nanotubes mixed with thermo-sensitive NIPAM was developed in order to enhance ADSC therapeutic efficacy. $2x10^6$ ADSC were seeded onto such systems and injected in a rat MI model. One week after treatment significant enhanced engraftment of seeding cells was detected when hydrogels were co-administered with the SCs in comparison to free ADSC administration. Moreover, LV-EF and FS, infarcted area and LV wall thickness were significantly improved in the ADSC-hydrogel group [69] providing evidence for the myocardial application of carbon nanotubes.

2.2.1.2. Hydrogels in protein-based therapies

The use of hydrogels for therapeutic protein delivery into the myocardium is quite recent. However, due to the liquid nature of the hydrogels, which facilitates cardiac administration, this DDS has rapidly attracted a great deal of attention.

Thermo-responsive hydrogels have been proved to favor positive remodeling and to improve cardiac function when combined with GF. For instance, a temperature-sensitive aliphatic polyester copolymer hydrogel made of PEG, N-hydroxysuccinimide and poly (δ-valerolactone) was conjugated with VEGF (40 ng) and intramyocardially injected in a rat MI model. No significant differences among groups were observed in any of the echocardiography parameters 7 days after treatment, but 35 days after treatment FS, EF, end-systolic elastance and end-systolic volume were significantly improved in the VEGF-hydrogel group compared to controls. Interestingly, conjugation of VEGF with the biomaterial prevented scar expansion and ventricular dilatation. Vascular density was significantly higher when VEGF was encapsulated inside the hydrogel [70]. Similar results were observed by Garbern J.C. *et al*. that entrapped biotinylated-FGF (5 μg) into a sharply pH-temperature-responsive injectable hydrogel system composed of a random terpolymer of NIPAM, propylacrylic acid and butyl acrylate. Hydrogels increased GF retention in the heart for 0-7 days when tested in a rat MI model. On the other hand, FS, regional myocardial blood flow, LV wall thickness and angiogenesis were significantly improved in the FGF-hydrogel group when compared to the other groups 28 days after treatment [71].

Apart from temperature sensitive hydrogels, other devices have been tested. For instance, Projahn D. *et al*. encapsulated Met-CCL5, a chemokine that inhibits neutrophil infiltration by competitive antagonism of CCL5 receptors, and SDF-1 in a

star-shaped poly(ethylene oxide-stat-propylene oxide) and linear poly(glycidol) hydrogel. In this strategy, polymer chemical modification led to different GF release profiles. Thus, Met-CCL5 (0.5 μg) was mixed with fast degradable hydrogel and SDF-1 (3 μg) with slow degradable one in order to optimize GF retention in the myocardium and to adjust the GF release to heart necessities over time. One day after injecting the systems in a mouse MI model, high levels of Met-CCL5 were detected in mouse sera, but this trend was not maintained 4 weeks after hydrogels injection. On the other hand, levels of SDF-1 remained constant due to the slow release. Regarding heart recovery, EF was significantly higher 4 weeks after treatment when both GF-hydrogels were administered. Regarding neovascularization, apoptotic levels and infarcted area size, they were significantly improved when SDF-1 was administered, alone or in combination with the other GF, suggesting that there was accelerated wound healing in these groups [72].

In another study, PEG-based hydrogels were formulated by mixing this compound with maleimide macromers, and the systems were pre-functionalized with RGD adhesion peptide. HGF and/or VEGF (both at concentration of 1 μg per injected hydrogel) were incorporated into the matrices, which were tested in a rat MI model. The chemical-sensitive precursor hydrogel solution was crosslinked into a hydrogel by addition of a cysteine-flanked protease-degradable peptide sequence. Thus, before administration, crosslinked agent was added and final solution was injected into the ischemic zone. Only when both GF were coadministered a significant cardiac function improvement could be observed 21 days after treatment with respect to the non-treated group. Significant increase in both vessel density and fibrosis and in c-kit$^+$ cells were observed after HGF-VEGF-hydrogel treatment [73]. Nevertheless some controversial results were observed in larger animal models when other PEG based hydrogels were employed. This was the case of the study by Koudstaal S. *et al*., who developed a pH-switchable supramolecular hydrogel with self-healing properties made of PEG and 2-ureido-4-pyrimidone (UPy). These systems were combined with insulin like growth factor 1 (IGF-1, 2 μg) and HGF (2 μg) and administered in a pig MI model. Although the dual GF-hydrogel combination resulted in improvements in LV end-systolic volumes, EF and formation of new capillaries in the infarct border zone one month after the injection procedure, no other benefits were detected. Interestingly, regarding CMC hypertrophy rate, although GF administration attenuated CMC degeneration, no significant differences were observed between the hydrogel group and the free GF administration group [74].

Another type of chemical-sensitive hydrogels was used by Wang T. *et al*. who employed their biocompatible PEG-based hydrogel solution, previously used as cell carrier (see [55]), as delivery system to administer EPO into rat MI model. In order to favor hydrogel formation, PEG solution and EPO solution dissolved with α-cyclodextrin were co-injected together. One month after treatment, animals that had received EPO dissolved in saline medium or incorporated into the hydrogel showed significant improvements in echocardiography parameters. However, significant infarct size reduction and apoptotic index, as well as increase in CD34$^+$ cell density and neovasculature formation were only detected in the EPO-hydrogel group indicating the benefits of this strategy [75].

Self-assembling peptides are nowadays attracting growing interest for protein delivery. For instance, RAD16-II peptide solution in combination with platelet-derived growth factor (PDGF, 4 and 8 μg) has been used to synthesize chemical depending hydrogels, which undergo gelation once peptide solution is mixed with sterile sucrose [76]. The highest dose system showed decreased CMC death and preserved systolic function 14 days after being injected in a rat MI model. Previous observations were correlated with a decrease in infarct size and induced PDGF receptor β expression and Akt phosphorylation in cardiomyocytes *in vivo* that indicated that CMC were protected by endothelial cells through PDGF-pathway. The same group performed a long-term study in which intramyocardial delivery of PDGF by self-assembling peptide hydrogel led to an improvement in cardiac performance for at least 3 months [77]. In order to prolong and slow angiogenic factors release, Guo H.D. *et al*. constructed a novel self-assembling peptide by attaching the heparin-binding domain sequence LRKKLGKA to the self-assembling peptide RAD16 encapsulating VEGF (100 ng). In a rat MI model EF, FS, scar size, collagen deposition, cell survival and microvessel density were significantly improved in animals treated with the novel hydrogel compared to VEGF-RAD16 hydrogel (without LRKKLGKA sequence) 4 weeks after treatment [78]. Segers V.F. *et al*. also observed positive results in cardiac recovery using RAD16-II peptides. In this case, SDF-1 variant resistant to protease degradation was encapsulated into hydrogels and administered in a rat MI model. Treatment resulted in significant enhancements on SC recruitment, improved cardiac function and capillary density 28 days after administration [79]. Interestingly, when animals were treated with the SDF-1 resistant variant, a better heart recovery was observed compared to the normal SDF-1 treated group, although no significant differences were reported.

Encouraged by RAD16-II peptide result, Kim J.H. *et al*. optimized the therapy by combining PDGF and FGF-2 in the same hydrogels. The systems were injected in a rat MI model and dual GF loaded hydrogels showed the smaller CMC apoptosis rate compared to the other groups. Infarct size and wall thickness followed similar significant trends 4 and 8 weeks after treatment. Interestingly, animals treated with PDGF-FGF-hydrogel showed similar vessel density to non-infarcted animals, suggesting an important angiogenic synergy between both GFs [80]. Dual GF delivery strategy for preserving cardiac function was also explored by Webber M.J. *et al*. in a mouse MI model, who loaded VEGF and FGF (10 ng of each GF per hydrogel) in heparin-binding-peptide-amphiphile hydrogels. VEGF-FGF-hydrogel treatment resulted in significantly improved LV contractility 30 days after administering the treatments [81].

Hydrogels made of semi-synthetic materials have also been explored as protein carriers for cardiac repair. He Y.Y. *et al*. used dextran in combination with hydrophobic poly (e-caprolactone)-2-hydroxylethyl methacrylate chain and thermo-responsive NIPAM forming thermosensitive hydrogels. 2.5 μg of high-mobility group box 1 (HMGB1, cytokine that attenuates cardiac remodeling after MI) were added per hydrogel, and then tested in a rat MI model. 24 hours after treatment administration, cardiac SC proliferation and differentiation were found to be significantly higher in HMGB1-hydrogel group compared to the other groups. One month later, HMGB1-hydrogel treated animals showed the greatest increase in EF and the lowest collagen deposition, with significant differences from all other groups. Nevertheless both HMGB1-hydrogel and free HMBG1 significantly increased arterial density in the peri-

infarcted area when compared to controls, but no significant differences were observed between these groups [82].

Hyaluronan is a natural polysaccharide which has been mixed with synthetic compounds to prepare hydrogels due to its excellent biocompatibility and biodegradability [83]. For instance, sodium hyaluronate was chemically modified with hydroxyethyl methacrylate to favor hydrolytic degradation, as in the work of MacArthur J.W. Jr. *et al*. A synthetic analog of SDF-1α was encapsulated at a concentration of 25 μg/50 μL and APS/TEMED was used for hydrogel gelation. These systems were injected intramyocardially in a rat MI model, where they proved to have significant benefits in improving echocardiography parameters such as EF, cardiac output and contractility when compared to controls. Loaded hydrogel also augmented capillary density. However, no significant differences were found between SDF-hydrogel and hydrogel groups regarding preservation of ventricular geometry and infarct size region, although both were significantly improved when compared to control groups [84].

2.2.2. Nanofibers

NFs are tridimensional, polymeric matrices with a network structure made of engineered fibers with diameters less than 500 nm (Figure 4). To date several biomaterials have been tested as potential NFs for inducing cardiac repair after MI. For instance, in the work of Castellano D. *et al*., collagen, poly(3-hydroxybutyrate), poly(e-caprolactone), poly-lactic acid and polyamide NFs were generated by electrospinning, being then transplanted into a rat MI model. Interestingly, poly(3-hydroxybutyrate) was the scaffold with the most beneficial reparative potential and positive remodeling capacity [85]. In another study polyester urethane urea NFs showed suitable mechanical properties and biocompatible characteristics, allowing cellular integration and endocardial endothelialization with minimal inflammation [86]. Thus, the evidences suggested that NFs result in positive outcomes for MI treatment.

2.2.2.1. Nanofibers in cell-based therapies

Since NFs are solid networks, cells can be entrapped within the polymeric matrix, augmenting their engraftment and survival. With this aim, Jin J. *et al*. combined MSCs with poly(lactide-co-ε-caprolactone) NFs. The systems, containing $1x10^6$ MSCs per NF construct, were sutured onto the epicardial surface over the infarcted region of a rat MI model. Four weeks after treatment, echocardiography showed that SCs administration, regardless the co-administration of NFs, resulted in LV dilation and improved EF compared with the control groups, and SCs survived and differentiated into cardiomyocytes. Only infarct area was significantly reduced in the MSC-NFs group compared to other groups [87]. More recently poly(ε-caprolactone) was mixed with gelatin to prepare NFs by electrospinning. $2x10^6$ MSC were seeded onto these hybrid scaffolds and transplanted into a rat MI model. Cells within the NFs were able to migrate towards the scar tissue, promoting new blood vessel formation at the infarct site. Consequently, 4 weeks after transplantation, the seeded NFs restricted the expansion of the LV wall, reduced the scar size and improved cardiac function

significantly compared to the other groups [88]. Other similar co-polymers such as poly-glycolide-co-caprolactone (PGCL) and polyglycolic-acid (PGA) have been used as synthetic and biocompatible NFs for myocardial implantation. Piao H. *et al*. seeded $2x10^6$ BMSC on PGCL-NFs and injected such systems into the epicardial surface in a rat MI model. Four weeks after implantation, BMSC-NFs group showed higher but no statistically significant migration of BMSC into the epicardial region, as well as a greater differentiation rate towards cardiomyocytes. Induction of neovascularization, reduced fibrosis, positive remodeling and ameliorated LV function were detected in BMSC-NFs treated group when compared to controls [89]. In the work of Ke Q. *et al*. PGA-NFs were combined with ESC ($5x10^4$), being then transplanted onto the surface of ischemic myocardium of infarcted mice. ESC-NFs treatment not only improved blood pressure and ventricular function, but also had significantly higher survival rates compared to all other groups eight weeks after treatment [90].

More recently, polyurethane (PU) has drawn attention due to its softness, elastic and biodegradation characteristics. In addition PU allows CMC to grow in organized layers matching physical and mechanical properties of the native tissue [91,92]. Thus, Blumenthal B. *et al*. seeded SM ($5x10^6$ cells) on such systems but, interestingly, they previously transfected the SM with DNA of VEGF, HGF, SDF-1, or serine-threonine protein kinase (Akt1). Their final constructs resulted in GF-producing myoblast-seeded PU NFs. After being sutured at the epicardial zone of infarcted rats, SM-NFs were found to be accepted by the host with no inflammatory reaction detected after 6 weeks. This was correlated with enhanced angiogenesis when SM were transfected with VEGF, HGF and Akt1, and with reduced infarction area when SM over-expressed SDF-1 and Akt1 or when SM were untransfected [93]. Two years later, in 2012, a couple of interesting studies were performed in the same direction. On one hand, von Wattenwyl R. *et al*. used VEGF-overexpressing myoblasts ($5x10^6$) seeded on PU-NFs [94]. On the other hand, Poppe A. *et al*. transfected SM ($5x10^6$) with HGF and then seeded them on the same DDS [95]. Apart from stimulating endothelial cell motility and enhancing angiogenesis, intramyocardial HGF secretion after ischemic injury was associated with less severe ventricular enlargement and with an improved cardiac function [96]. In both cases, the seeded scaffolds were intramyocardially transplanted in infarcted rats, and six week later hemodynamic parameters and histological analysis were performed. The administration of HGF-overexpressing SM in PU-NFs resulted in an increased capillary density on the infarcted and peri-infarcted regions. Nevertheless, statistical analysis showed no significant changes in infarct size between groups. Regarding cardiac function, only the HGF overexpressing SM-NFs treated group showed a significant improvement from baseline at the end of the study [94,95]. These results are in correlation with the study of Giraud M.N. *et al*., who studied how myoblast-seeded PU-NFs could prevent cardiac dysfunction. Highly porous NFs with SM ($5x10^6$) were attached to the outer myocardial scar surface of MI rats. Only SM-NFs significantly prevented progression towards heart failure 9 months after treatment compared to the other groups, but this effect vanished 12 months after treatment. Interestingly, the systems were correctly incorporated into the cardiac tissue as new-formed vessels were formed inside the DDS [97].

2.2.2.2. Nanofibers in protein-based therapies

The use of NFs as protein delivery systems is a relatively new field and only a limited number of studies have explored their application in cardiac repair. For instance, Wang Y. *et al*. formulated poly(lactic-co-glycolic acid) (PLGA) NFs loaded with FGF (15 μg). This system significantly enhanced neo-vascular formation, blood flow, FS and the number of proliferating cells 6 weeks after implantation in a mini-swine MI model [98]. In another approach, poly-vinyl-alcohol (PVA) was combined with dextran to form solid injectable NFs for the delivery of FGF (100 μg). These systems were tested in a large ovine MI model. FGF-NFs were sutured to animals' epicardium, showing a sustained release of FGF that strongly stimulated angiogenesis and increased wall thickness index in the infarcted myocardium 2 months after treatment. The NFs also significantly attenuated the increase in LV end-systolic diameter, but did not improve cardiac function [99]. Positive results were reported by Zhang G. *et al*., who reported that PEGylated fibrin NFs loaded with SDF-1 (100 ng), when injected in a mouse MI model, significantly increased myocardial recruitment of c-kit$^+$ cells compared to controls two weeks after treatment. Enhanced stem cell homing was maintained at 28 days, when LV function was significantly improved in comparison with the controls [100]. More recently, our group prepared smooth polymeric NFs of stat-modified PLGA to deliver NRG to the heart. *In vivo* biocompatibility studies demonstrated that NFs were present in the heart 3 months after administration and a constructive tissue remodeling was observed indicating good incorporation into the organism [101]. In ongoing studies, the efficacy of this system is being evaluated.

2.2.3. Nano and micro-particles

DDSs based on nano and microparticles (NPs and MPs, respectively) have shown great potential to improve the treatment of many diseases, including cardiovascular disorders. They are solid particles in the nanometer of micrometer size range in which the active principle is dissolved, entrapped, encapsulated or adsorbed [102]. There is a long list of materials that can be used to prepare particles of a desired size. Depending on the raw materials employed, drug release profiles, particle degradation and location of the particles can be controlled. Generally, NPs and MPs suffer faster degradation processes than hydrogels or NFs. This higher biodegradability allows their total elimination from the biological tissues avoiding chronic inflammation responses. Together, these characteristics make particles one of the more versatile DDS on the market [103].

2.2.3.1. Nano/microparticles in cell-based therapies

Regarding strategies based on the use of SCs combined with NPs, covalent coupling, adsorption and internalization of NPs inside cells have been used [104]. It is important to note that NPs were not used for encapsulating or conveying SCs on their surface due to their relatively small size, but for augmenting their circulation time, targeting cells towards specific tissues, improving SCs function *in vivo* [104], modifying cell behavior [105], delivering biomolecules and genes and for diagnostics and imaging methods [106].

On the other hand, MPs can be formulated to encapsulate [107,108] or to convey [37,109] cells on their surface. Nevertheless, although alginate and matrigel MPs have reported promising results [107,108], only few studies have been performed with MPs made of synthetic materials. In a recent study human amniotic fluid SCs ($1x10^6$) were encapsulated inside PLGA porous MPs of about 250 µm. The efficacy of these systems was tested in a rat MI model, showing that animals treated with SCs-MPs had a significantly increased capillary density and positive remodeling, which resulted in an improved cardiac function 4 weeks after treatment compared to other groups. SCs were clearly retained at the site of injection and were differentiated towards cardiomyogenic and angiogenic lineages [110]. In another study, Penna C. *et al*. demonstrated that PLGA-MP enhanced MSC survival and regeneration in the hostile environment of post-ischemic tissues [109].

2.2.3.2. Nano/microparticles in protein-based therapies

Encapsulation of proteins in NPs/MPs is one of the approaches that have been most extensively investigated to protect therapeutic molecules against *in vivo* degradation and to release drugs in a controlled manner [36,102]. Apart from their well-established efficiency as DDS, their surface modification possibilities [111] have given NPs and MPs an interesting therapeutic potential. In fact, active targeting [112] is a very common strategy that has given NPs a particular interest for intravenous administration [113], since they can pass through the microcirculation easily [114] and they are not very vulnerable to immune clearance [115,116], finally reaching heart tissue. Nevertheless, any targeted NPs formulation have been clinically approved yet [111]. Regarding MPs, their relative large size makes their intravenous administration impossible without causing undesired side effects. Consequently, in the few *in vivo* studies developed so far, local delivery of NPs and MPs remains the most common way of administration. Along these lines, Sy J.C. *et al*. developed poly(cyclohexane-1,4-diyl acetone dimethylene ketal) MPs (polyketal-MPs) and PLGA-MPs of around 20 µm encapsulating SB239063 (0.5 mg), which were intramyocardially injected in a rat MI model. SB239063 molecule is an inhibitor of apoptotic protein p38, which is related to the progression of cardiac dysfunction. Both types of MPs allowed an *in vivo* sustained release of SB239063 over at least 7 days. Authors observed significant less fibrosis and improvements in cardiac function 21 days after treating MI rats with the SB239063-polyketal-MPs group but, interestingly, no significant improvements with respect to controls were detected in the SB239063-PLGA-MPs animals [117]. Based on these results, the same group also synthesized superoxide dismutase 1 (SOD1) polyketal-MPs (protein:polymer ratio of 0.05) (10 µm). SOD1 is a protein with antioxidant effects that has proved to favor infarct size reduction after a MI event [118]. When injected intramyocardially in a rat MI model, MPs were detected for up to 10 days in the myocardium. Superoxide levels were decreased in animals treated with SOD1-MPs when compared to controls, and the same significant trend was observed in CMCs apoptosis ratios. Three days after treatment, improvements in FS were only observed when SOD1 and SB239063-MPs were co-injected, suggesting the need of multiple therapeutics dosage to combat the different phases of the disease [119]. In order to enhance NPs uptake by CMC, SB239063-polyketal-NPs were covered with the sugar N-acetyl-D-glucosamine (GlcNAc) [120] and their efficacy was tested in a rat MI model.

The number of apoptotic CMCs was significantly lower in the GlcNAc-SB239063-NPs group compared to other groups 24 hours after treatment. This result was confirmed by an uptake study, where GlcNAc-polyketal-NPs were clearly more captured by CMCs than non-coated NPs. Three days after treatment echocardiography analyses showed that only rats that received loaded-NPs had a significant reduction in infarct size/area-at-risk and an improved FS [121].

Due to their well-established *in vivo* biocompatibility, safety and FDA approval, polyesters like PLGA are widely used in cardiac tissue engineering. One interesting example is the study by Chang M.Y. *et al*., where PLGA-NP of 60 nm, 200 nm and 1 µm were synthesized containing different concentrations of IGF-1. When intramyocardially injected in a MI mice model, 24 hours after treatment IGF-1 was significantly more in IGF-1-NPs treated group compared to free IGF-1 administration. IGF-NPs treated animals also showed a significant reduction in infarct size and number of apoptotic CMCs and improved LV-EF 21 days after treatment compared to free IGF administration and non loaded NPs. Finally, the authors reported that 60 nm NPs were most effective in binding IGF-1 and consequently preventing CMCs apoptosis [122]. Our group has also examined the feasibility of using PLGA-MPs encapsulating therapeutic proteins to promote cardiac regeneration. First, Formiga F.R. *et al*. prepared PLGA-MPs (5 µm) containing VEGF (35 µg per 50 mg of MPs) by solvent extraction/evaporation method using TROMS technology. This technology based on double emulsion and solvent evaporation methods allowed them to encapsulate labile proteins without altering their nature properties and bioactivity [123]. The systems were administered via intramyocardial injection in a rat MI model. One month after treatment, PLGA-MPs were present in the myocardium, and significant increments in angiogenesis and arteriogenesis in the infarct and peri-infarct areas of the injured hearts in the VEGF-MPs group were detected in comparison to controls. The increased revascularization of the tissue translated into a beneficial effect in the remodeling processes, with a significantly greater thickness of the LV wall in the VEGF-MPs treated animals in comparison to the rest of the groups [123]. In addition, Simón-Yarza T. *et al*. combined both angiogenic and antioxidant drugs to establish potential synergistic effects. With this aim, PLGA-MPs of 5 µm containing VEGF (50 µg per 50 mg of MP) and PLGA-NPs (150 nm) encapsulating Coenzyme Q10 (CQ10, 1.5 g per 3 g of NPs) were formulated. CQ10 is known due to its antioxidant and cardioprotective roles [124]. The efficacy of VEGF-MPs and CQ10-NPs was studied in a rat MI model, where MPs were intramyocardialy injected and NPs were administered orally. Separately, both treatments demonstrated significantly increased EF three months after administration when compared to the other groups. That was correlated with a highly significant increase in the number of capillaries in the infarct and peri-infarct areas. Interestingly, CQ10-NPs showed better outcomes than commercial CQ10, what was attributed to the ability of NPs to improve oral bioavailability and to the sustained release of the encapsulated CoQ10. Unfortunately, combined treatment failed to offer synergy, and no EF improvements could be observed [125]. In another study, we successfully delivered FGF-1 and NRG-1 to the ischemic tissue using PLGA MPs (5 µm), administering a final amount of 1740 ng of FGF and/or 1300 ng of NRG in treated animals. Three months after treatment, global cardiac function, infarct size, fibrosis, revascularization and cardiac stem cell recruitment were significantly increased in GF-MPs treated groups (FGF, NRG or FGF/NRG) when compared to controls [126] (Figure

5). Our study is providing very useful data regarding the underlying mechanisms contributing to the beneficial effects of this therapy, especially those linked to endogenous regeneration, which might be very useful for the design of novel cardiac repair approaches. As a prerequisite for clinical application, we next determined the long-term therapeutic effectiveness and safety of this therapeutic strategy in a pre-clinical large animal model of myocardial infarction (mini-pigs) demonstrating that cytokine delivery MPs are able to restore cardiac function [35]. This technology could soon be translated to humans.

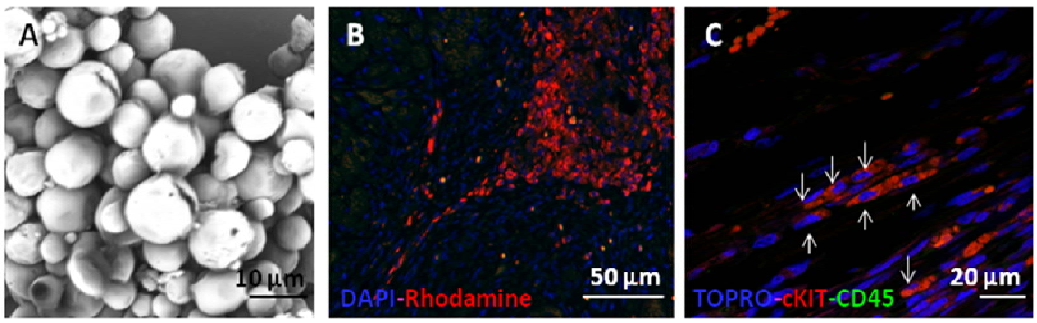

Figure 5: NRG-PLGA MPs. A) Scanning electron microscopy of NRG-PLGA MPs. B) Tissue retention of fluorescent PLGA MPs 1 month after intramyocardial injection in a rat MI model. C) Cardiac progenitor cell recruitment 1 week after intramyocardial injection of NRG-PLGA MPs.

Finally, Oh K.S. *et al*. prepared semi-synthetic NPs made of a lecithin core and a Pluronic F-127 coating with a Capryol 90 hydrogel system in order to achieve a stable localization of VEGF-NPs at the ischemic area. In this case, VEGF (5 µg) was encapsulated inside the natural core of NPs and final systems (270 nm) were epicardially injected in a rat MI model. Although both VEGF-NP and VEGF-NP-hydrogel resulted in significantly improved capillary density, significantly higher cardiac function was observed in VEGF-NP-hydrogel group compared to all other groups [127].

2.2.4. Liposomes

The latest example of biomaterial-based DDSs reviewed here are liposomes. Liposomes are sphere-shaped vesicles consisting of one or more pohspholipid bilayres (Figure 4) [128]. The liposomal drug Doxil was the first lipid system to be used in clinical practice in 1999. Although nowadays they are used in other diseases[129,130], there are still no liposomal formulations approved for human use for the treatment of cardiovascular disease.

Concerning liposomes, active targeting has been deeply explored, and surface-attached targeting molecules have been employed for preparing liposomes used to target MI, constituting a promising approach for heart therapy [131]. Thus, immunoliposomes (ILs) presenting phosphatidylserine (PS) on their surface can be easily recognized by macrophages, which are relevantly concentrated in the cardiac tissue after MI due to the inflammatory process, providing specific accumulation of

targeted ILs in the damaged heart. This strategy has been used by Harel-Adar T. *et al*. who synthesized PS-ILs. Firstly, the systems were intraperitoneally injected in rats, and 3 hours later, the peritoneal cells were analyzed. The state of macrophages changed from pro-inflammatory to anti-inflammatory. That was translated in significantly higher levels of anti-inflammatory cytokines on the peritoneal lavage fluids in treated animals compared to controls, which confirmed the previous result. When this was translated to a rat MI model, the same protective trend was observed. Interestingly, PS-ILs induced cardiac macrophages to secrete anti-inflammatory cytokines 3 days after treatment, which is 1 day earlier than under normal conditions. The treatment also promoted angiogenesis, prevented ventricular dilatation and remodeling, and small scars were detected in comparison with control groups [132].

Concerning cell therapy and liposomes, no results have been published yet, so the following section will be focused on liposomes for protein delivery.

2.2.4.1. Liposomes in protein-based therapies

A large number of the physicochemical properties of liposomes [133] have been explored for active targeting of therapeutic drugs to myocardial ischemic regions [134]. PEGylation strategy is used to improve permanence-time and to reduce the opsonization process of systems administered in the blood [135,136], consequently increasing liposome therapeutic efficacy [137]. Regarding heart targeting, one adhesion molecule that is up-regulated on endothelium in response to ischemia and inflammation is P-selectin [138]. In the work of Scott R.C. *et al*., PEGylated phosphatidylcholine/cholesterol liposomes were synthesized and incubated with IgG2a mouse antibody to rat P-selectin. VEGF (0.12 g/kg animal weight) was encapsulated in such systems and administered *via* tail vein immediately after induction of MI in rats. ILs were selectively accumulated in the myocardial infarct region [139], allowing targeted VEGF delivery to post-MI tissue, which resulted in significant increase of FS and improved systolic function. These functional improvements were associated with an increase in the number of vessels in the MI region of treated animals [140]. Similarly, Wang B. *et al.* developed anti-P-selectin conjugated ILs to target the delivery of VEGF to the heart, which significantly improved vascularization and cardiac function [141]. Using other approach, Yamada Y. *et al*. prepared Sialyl Lewis X molecule (SLX) ILs (100 nm) encapsulating EPO [142]. SLX is a carbohydrate present in the leucocytes membrane known for interacting with selectin cell-adhesion proteins and to play a vital role in cell-to-cell recognition processes [143]. SLX-EPO-ILs were intravenously administered in a rabbit MI model (2,500 IU of EPO/kg body weight). Only ILs but no non-targeted liposomes were selectively accumulated at the border area of the infarcted myocardium, significantly increasing EPO levels in the heart 48 h after treatment. LV remodeling, EF, FS and reduction on MI size were significantly improved in the SLX-EPO-ILs group when compared to controls. Similar results were observed for the number of $CD31^+$ microvessels and for EPO receptor expression [142].

3. EMERGING TISSUE ENGINEERING STRATEGIES FOR HEART REGENERATION AFTER MYOCARDIAL INFARCTION

The combination of cells or protein with biomaterials has proved to be effective in preclinical animal models of MI. In brief, regarding cell therapy, it has been possible to enhance cell viability and engraftment. Biomaterials have enabled cells to assemble into effective tissue substitutes that may restore cardiac functions and structure. Concerning protein therapy, the use of DDSs has allowed researchers to protect growth factors against *in vivo* degradation and to achieve a controlled release over time, favoring important processes during cardiac healing such as angiogenesis or SC differentiation towards cardiac lineages. Moreover, SCs can directly benefit from the action of therapeutic growth factors. For instance, SCs depend on growth factor for correct survival and differentiation (Figure 6). In addition, SC paracrine secretions together with therapeutic growth factors may achieve a better regenerative effect. Thus, some authors have investigated the combination of both cellular and protein therapies together with biomaterial-based delivery systems. This integrated approach, known as the tissue engineering triad, has attracted considerable attention over the past years (Figure 6).

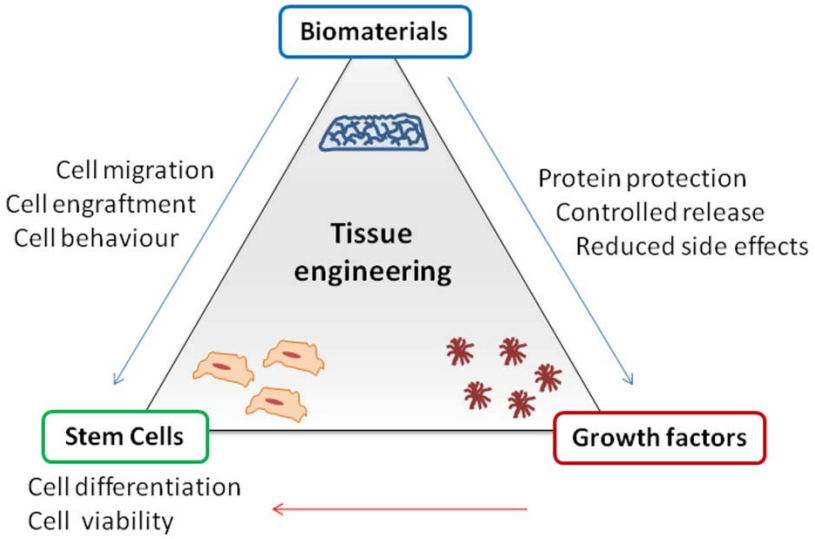

Figure 6: Tissue engineering triad, with the benefits that each element (growth factors, SCs and biomaterials) give to other element.

Since tissue engineering is a novel approach only a few studies have been published so far, although showing interesting results. In the first study in 2005, a bioengineered NFs scaffold made of polyglycolic acid succeeded in incorporating BMSC ($1x10^7$) and FGF (0.2 µg). When such systems were transplanted in a rat MI model, cells were detected inside the scaffold 4 weeks after implantation, and NFs were absorbed by the host tissue indicating good incorporation into the organism. Global cardiac function and capillary density were significantly improved in BMSC-FGF-NFs treated animals when compared to BMSC or FGF loaded NFs groups [144]. Similarly, our group investigated the feasibility of using NRG-releasing PLGA-MPs (20 µm diameter, 1.8 µg NRG/mg of MP) combined with ADSC ($2.5x10^5$ or $5x10^5$) as a multiple growth factor delivery-based tissue engineering strategy for implantation in the infarcted

myocardium [37]. ADSC-NRG-MPs proved to be compatible with intramyocardial injection in a rat MI model and systems were present in the peri-infarcted tissue 2 weeks after implantation [37]. Efficacy studies are currently being performed. Apart from those polymers, other synthetic materials have been employed for generating injectable DDSs. The group of Kraehenbuehl T.P. *et al*. formulated a three-dimensional metalloproteinase-sensitive PEG-based hydrogel, and used such systems to deliver thymosin β4 (Tβ4, 2.5 μg) in combination with ESCs ($6.6x10^6$) and smooth-muscle stem cells ($3.3x10^6$) in ischemic injuries of a rat MI model. Tβ4 protein activates the survival kinase Akt, protects cardiac muscle form death after ischemic damage and promotes angiogenesis, making it an interesting molecule for cardiac regeneration [145]. Thus, the cell seeded-Tβ4-hydrogels effectively preserved contractile performance 6 weeks after myocardial infarction and attenuated LV dilation compared to controls and to the Tβ4-hydrogels treated group [146]. Neovascularization and infarct size were also significantly improved in cell seeded-Tβ4-hydrogels and Tβ4-hydrogels groups compared to controls.

Concerning full synthetic biomaterials, self-assembling peptide RAD16-II has been used to create injectable hydrogels incorporating IGF (approximately 1 ng) in combination with CMC ($1x10^6$) [147] or CPC ($1x10^5$) [148] for cardiac repair. In both studies the administration of cell-seeded-IGF-hydrogels significantly improved the recovery of myocardial structure and function in rats one month after treatment. Apoptosis was also reduced regardless of the cell type, but a reduced infarct size and increased capillary density were only reported when CPC were co-injected with IGF [148]. In any case, the presence of IGF resulted in a protective environment that favored SC proliferation. In other study using the same RAD16-II peptides, Dubois G. *et al*. compared the efficacy of skeletal myoblasts (SMs) and PDGF therapies to SM-PDGF tissue engineering in a rat MI model. Significantly greater angiogenesis was observed in all GF-treated groups compared to controls one month after treatment. However, this was not correlated with an improved cardiac function. In fact LV function was not improved in either of the treated groups compared to controls at the same time point. The lack of functional improvements observed *in vivo* was explained by an *in vitro* SM viability study. Authors concluded that specific tailoring of the biomaterial to the cell type is required for correct cell survival [149].

The combination of synthetic and natural biomaterials is common in tissue engineering, and relevant promising results have been obtained. For instance, semi-synthetic hydrogels made of PEGylated fibrin biomatrix efficiently bound HGF and entrapped BMSC ($5x10^5$). After administration in a mouse MI model, the systems allowed significant improvements in cell prevalence at the injection site for at least 4 weeks, compared to free cell administration. Interestingly, in BMSC-HGF-hydrogel treated animals, cell retention was accompanied by the lowest levels of apoptosis and the highest LV function recovery among all the groups, confirming that tissue engineering was more effective than protein or cell therapy alone [150].

In order to obtain a system inspired by tissue-specific niches able to mimic the real biological process of heart healing, several DDSs have been combined. Thus, in the work of Holladay C.A. *et al*. MSC were seeded onto semi-synthetic hydrogels of collagen, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide and N-hydroxysuccinimide. 2 μg of interleukin-10 (i10), the most potent anti-inflammatory cytokine, was

incorporated into dendrimer polyplexes and incubated with the hydrogels. Four weeks after treating MI rats with such systems, SC retention and FS were found to be significantly improved in animals which received MSC-i10-systems compared to the rest of the groups. Improved function was associated with increased infarcted wall thickness, decrease of cell death and a change in macrophage markers from mainly cytotoxic in the MSC-hydrogel group to mainly regulatory in MSC-i10-system treated group, confirming the success of tissue engineering over cell therapy [151]. Using another strategy, Miyagi Y. *et al*. combined both NFs and hydrogels. Thus, authors first synthesized a gelfoam/poly-(ε-caprolactone) NFs construct. Then, BMSC ($1x10^6$), stem cell factor and/or SDF (30 ng of each one) were incorporated in a polymeric temperature-sensitive hydrogel made of valerolactone and PEG. MI was induced in rats, and after two weeks NFs were transplanted covering the infarcted area. At the same time, hydrogels were injected next to the NFs. Four weeks after treatment, those animals treated with NFs and SC-GF-hydrogels showed a significant global cardiac function improvement compared to animals treated only with the NFs. Although NFs in combination with SC-GF-hydrogel treatment resulted in better heart recovery compared to controls, no significant differences were observed when compared to animals that had received NFs and SC-hydrogel or GF-hydrogel. Finally, neovascularization and wall thickness were enhanced in all treated animals compared to controls [152].

3.1 Challenges ahead

The above examples represent some of the ways in which tissue engineering strategies are being investigated to address cell and protein hurdles. Interestingly, several synthetic biomaterial-based DDS have been explored although no one has proved to be better than the others. In any case, if we want to reach clinical applications, new techniques for treating MI must not be more invasive than the existing cardiac procedures. Concerning this aspect, hydrogels, NPs, MPs, and liposomes achieve this goal, and can be administered by trans-endocardial injection or *via* catheters. On the other hand, NFs need to be attached to the pericardium, so a more invasive administration technique is required. However, it is highly desirable that biomaterials should provide satisfactory mechanical support to the infarcted heart, in order to favor functional recovery of the damaged organ [33]. In this sense, NFs have proved to be able to contribute more efficiently to the heart's mechanical properties than other DDS. However, given the intricate anisotropic mechanical behavior of myocardium, it is not easy to produce a biomaterial that responds to mechanical stresses in a way that is similar to the heart itself. In this regard, PU seems to be the most promising biomaterial [91,92]. Another recommendable characteristic for DDS in cardiac repair is the ability to mimic the natural heart microenvironment [153]. This way, biomaterials are used as an alternative to extracellular matrix, being NFs and hydrogels the DDS that reproduce natural conditions in the best way possible. Taking all of this into account, we can say that the search for the optimum biomaterial-based delivery system still continues and further research is this area is guaranteed.

We cannot forget that the mammalian heart is a complex organ composed of a heterogeneous cell population. Consequently, the potential of a long list of SCs and GFs for regenerating the infarcted heart tissue has been investigated so far. Tissue

engineering has proved to be useful in regenerative medicine in terms of high viability and long-term engraftment of cells. In addition, cardiac repair and regeneration is favored by effective delivery of therapeutic GFs. Nonetheless, although all tissue engineering strategies regardless of the therapeutic agent employed have enhanced myocardial functional, the repair mechanisms remain unclear at the moment. It is still unknown whether the repair of the infarcted heart is caused by the functional activity of the cells or by structural changes brought by biomaterials or proteins. Therefore myocardial tissue engineering approaches have to be developed considering both cell and GF requirements of the heart for successful cardiac recovery. In addition, functional integration between the graft and the host tissue, in both electromechanical and vascular terms, still remains a major challenge that must be considered when designing new cardiac tissue engineering approaches [154]. The establishment of well-defined protocols and the optimization of the synergies between the different cells and GFs are required before clinical applications can be attained. In fact, tissue engineering is still at the development phase and the only clinical trial evaluating a tissue engineering strategy is the one called ALCADIA. In this ongoing trial CPCs and FGF are being combined in a gelatin hydrogel to treat ischemic cardiomyopathy (Clinicaltrials.gov identifier NCT00981006). Thus, all of these promising results should be considered preliminary, and further studies are needed to confirm the possible benefits of myocardial tissue engineering.

4. CONCLUSIONS AND FUTURE PROSPECTS

New contributions to the advancement and optimization of classical treatments for MI have allowed a reduction in the number of death due to this pathology over recent decades. However, complications deriving from MI remain a big problem. Therefore, new strategies have been investigated to overcome such limitations, and the ones that have shown the most promising results so far are cell and protein therapies [14,15]. As this review has illustrated, both of these have encountered various challenges when tested in clinical trials, related to the low cell engraftment and the rapid degradation of therapeutic proteins once they are administered. Fortunately, it seems that nowadays we are close to reaching their full potential by combining them with biomaterials. Thus, this review has also demonstrated the relevance of biomaterials in the repair and regeneration of the damaged heart. Currently, synthetic hydrogels, NFs, NP, MP and liposomes are being investigated in depth in cardiac repair, in combination with cells and proteins. The capacities of these DDS to increase cell survival and engraftment, and to protect and control GF release are the main reasons for their success. However, the type of material, cell and GF sources, timing, dose and injection technique are still uncertain, and further investigation is mandatory in order to achieve the best patient outcomes. The current challenge is to establish a perfect combination of three components: biomaterials, cells and proteins. Tissue engineering is a rapidly evolving discipline. In fact, it is expected that in the next 10-20 years, these therapies will account for more than half of the new drugs introduced on the market [155]. Great advances have been made in the last few years, although there are still several aspects to improve and current results should be considered preliminary. In the future, MI treatments will surely represent an amazing challenge in terms of biomaterials and delivery systems with the final goal of providing many benefits to MI patients.

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